PHYSIOLOGY OF THE PHENOLS

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HARRY DUBIN

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PHYSIOLOGY OF THE PHENOLS.*

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INTRODUCTION.

The work of Baumann, Brieger, Salkowski, and others has seemingly solved the problem of the origin of phenols in urine. It is clear that the urinary phenols arise from intestinal putrefaction, and that they are derived from the tyrosine portion of the protein molecule. However, with the exception of the work of Folin,[1] little has been done—due probably to the lack of a suitable method—to determine (1) the extent to which phenols may be formed under both normal and pathological conditions, and (2) the relationship between the free and conjugated phenols. It was with these objects in view that the present work was undertaken.

HISTORICAL.

The literature has to a certain extent been reviewed by Folin,[1] so that it will be necessary only to record the results of some recent investigations.

The elimination of phenols is increased by absorption from wounds and abscesses.[2]

The relative ability of the various organs to dispose of phenol is as follows: liver, kidney, muscle, brain, and blood; *i.e.*, the liver has the greatest capacity for conjugating phenols, while the blood has the least. Only the epithelia of the intestinal tract give results comparable to those obtained with the liver.[3] The conjugating function of the liver is not much impaired by disease.[3]

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* Dissertation for the degree of Ph.D., University of Pennsylvania, 1916.

[1] Folin, O., and Denis, W., *J. Biol. Chem.*, 1915, xxii, 309.

[2] Hammarsten, O., Physiological Chemistry, New York, 1911, 689.

[3] Herter, C. A., and Wakeman, A. J., *J. Exp. Med.*, 1899, iv, 307.

On starving, the phenols, after an initial fall, increase considerably.[4] Müller[5] reported the case of a man who showed an increase of 155 mg. of phenol on the 9th day of starvation. This was held to be due to putrefaction of intestinal secretions. If, while an animal is starving, phlorhizin be given, there is an increase in the output of phenols, but not until sugar makes its appearance.[6] It is the increased protein breakdown, rather than intestinal putrefaction, that accounts, under these circumstances, for the increase in the elimination of phenols. Lewin[6] observed in rabbits an increase from 6.91 mg. of phenol to 16.35 mg. of phenol per day, on giving 0.8 gm. of phlorhizin subcutaneously. In man there was an increase of two or three times the original amount of phenol upon administering 0.25 gm. of phlorhizin *per os* or subcutaneously.

It is well known that if a measured amount of phenol be ingested it cannot be recovered quantitatively in the urine, and it is believed that the missing fraction is burned. Tauber[7] fed phenol to a dog and found that as the dose was decreased, the amount of phenol oxidized increased. Thus, he found no phenol in the fore-period, either in the urine or in the feces. On feeding 0.24 gm. of phenol in water *per os*, he found 110 mg. of phenol in the urine, and only 9 mg. in the feces. In other words, about 53 per cent of the ingested phenol was oxidized in the body through oxalic acid to carbon dioxide. Heffter[8] states that after feeding phenol, there is an increase in phenolsulfuric acid but not in free phenol. On the other hand, Reale[9] reports that poisoning with large amounts of phenol results in the presence of free, as well as combined, phenol in the urine.

Jonescu[10] found that after feeding *p*-cresol to dogs, kept on a diet of horse meat, only about 25 per cent of the amount ingested is eliminated, and that the elimination is complete in 24 hours. Taking an average of nine persons, Siegfried and Zimmerman[11] showed that of the total phenols eliminated, 58.1 per cent is *p*-cresol, and 41.9 per cent is phenol. They[12] found also that *p*-cresol, fed to dogs, is largely changed to phenol. Thus, on feeding 0.5 gm. of *p*-cresol with 0.5 gm. of sodium bicarbonate on 4 successive days, 32 per cent of the total amount fed was recovered in the urine. Similarly, on feeding 0.8 gm. of *p*-cresol, 48 per cent was recovered.

The occurrence of the phenols in the urine upon disinfection of the intestine is shown in an experiment carried out by Baumann.[13] A dog

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[4] Herter, C. A., Chemical Pathology, Philadelphia, 1902, 425.

[5] Müller, F., *Berl. klin. Woch.*, 1887, xxiv, 405, 436.

[6] Lewin, C., *Beitr. chem. Phys. u. Path.*, 1902, i, 472.

[7] Tauber, E., *Z. physiol. Chem.*, 1878–79, ii, 366.

[8] Heffter, A., *Ergebn. Physiol.*, 1905, iv, 242.

[9] Reale, E., Abstr., *Centr. klin. Med.*, 1891, xii, 487, quoted from *Jahresber. Tierchem.*, 1891, xxi, 401 (orig., *Gaz. clin.*, 1890, i, 2).

[10] Jonescu, D., *Biochem. Z.*, 1906, i, 399.

[11] Siegfried, M., and Zimmerman, R., *Biochem. Z.*, 1911, xxxiv, 471.

[12] Siegfried and Zimmerman, *Biochem. Z.*, 1912, xlvi, 210.

[13] Baumann, E., *Z. physiol. Chem.*, 1886, x, 123.

receiving only water for 2 days was given 2 gm. of calomel on the 2nd day. As on the 4th day ethereal sulfates were still present in the urine, 2 gm. of calomel were again given. The urine of the 2 following days was free of ethereal sulfates, and gave no test for phenol or indole. On the 6th day, the dog received 5 gm. of tyrosine, but no formation of ethereal sulfates resulted; neither was there an increase of hydroxy acids.

Wohlgemuth[14] fed 8 gm. of tyrosine to a rabbit and was able to recover a little less than 2 gm. of it. Dakin[15] reports no increase of phenolic substances after giving tyrosine or phenylalanine. After feeding tyrosine to cats, he recovered by crystallization from the urine very small amounts of tyrosine. He found also that with smaller doses no unchanged tyrosine could be recovered in the urine. Brieger[16] could find no tyrosine in the feces or urine after giving 20 gm. of tyrosine to a man weighing about 50 kilos. He did, however, find an increase in the phenols, and although the patient was constipated for 2 days after taking the tyrosine, Brieger held that the increase in phenols could not be due to constipation alone. Brieger's figures follow:

Day.	Phenol. *gm.*
1	0.0159
2	0.0225
3	0.0223
4	0.0182
20 gm. of tyrosine ingested in two portions.	
5	0.0493
6	0.1576
7	0.0851
8	0.0609
9	0.0348

Results obtained with animals and with man have led Folin[1] to conclude that the excretion of total phenol products in the urine appears to be much greater than is indicated by the phenol figures previously recorded in the literature. He shows further that the phenols are not quantitatively converted into conjugated phenols, so that the detoxication process involved in such conjugations appears to furnish only a partial protection against the toxic effect of the phenol products formed by putrefaction in the intestinal tract. Finally, the total phenol excretion tends to vary directly, but not proportionally, with the protein intake.

Some of the work described in this summary has been confirmed by our findings, while some has not. The value of our figures lies in the fact that

[14] Wohlgemuth, J., *Ber. chem. Ges.*, 1905, xxxviii, 2064.

[15] Dakin, H. D., *J. Biol. Chem.*, 1910–11, viii, 28.

[16] Brieger, L., *Z. physiol. Chem.*, 1878–79, ii, 241; 1879, iii, **134**.

they have been obtained by a more accurate method, that of Folin,[17] which permitted not only a definite quantitative determination, but also the study of the relationship between the free and the conjugated phenols.

Methods.

For phenols, free and conjugated, the technique of Folin[17] was used, bearing in mind the following precautions: (a) In determining total phenols, a few glass beads should be used to prevent the liquid in the test-tube from over-boiling, during the heating just prior to placing the tube in the boiling water bath. (b) The phosphotungstic phosphomolybdic acid reagent is prepared by boiling the various chemicals with 750 cc. of water instead of 75 cc., the amount stated in Folin's description, which was an error. (c) The stock phenol solution from which the standard is prepared holds its strength for a long period of time, whereas the standard phenol solution itself deteriorates, and should therefore be prepared freshly every 4 or 5 days. (d) The color obtained with the standard solution is not absolutely blue, but has a greenish tinge. This defect is remedied by filtration, without in any way affecting the colorimeter reading. Therefore, after standing the required 20 minutes, the standard is filtered through inexpensive coarse filter paper,[18] and the colorimeter readings are made at once.

With these precautions we have found the method accurate, rapid, and easy of application.

EXPERIMENTAL.

In this investigation an effort has been made to determine the behavior of the animal body, with respect to the formation and elimination of phenols, under both normal and pathological conditions. Dogs were chosen as being best suited for the work. After a period of normal observation, the animals were operated upon,[19] and the desired pathological conditions produced. All operations were done under ether anesthesia. In all, eight operations were performed, two each of: (*1*) *Eck fistula*; (*2*) *intestinal obstruction*; (*3*) *exclusion of bile from the intestinal tract*; (*4*) *exclu-*

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[17] Folin and Denis, *J. Biol. Chem.*, 1915, xxii, 305.

[18] Arthur H. Thomas Co., No. 27756, 125 mm.

[19] For the operative work, I am indebted to Dr. Max Minor Peet, of the Department of Surgical Research.

sion of pancreatic juice from the intestinal tract. The technique employed in each operation was as follows:

1. Eck Fistula.—An artificial anastomosis between the portal vein and the inferior vena cava, with ligation of the portal vein at the hilus of the liver, was made according to the technique described by Peet.[20]

2. Intestinal Obstruction.—Almost complete obstruction of the ileum was obtained about 6 inches from the ileocecal valve by detaching from the external sheath of the rectus a strip about ½ inch wide and 1 inch long, passing it through the mesenteric attachment around the gut, overlapping, and suturing the two ends of the fascia firmly together.

3. Exclusion of Bile from the Intestinal Tract.—The common bile duct was tied in three places, cut between the ligatures, and part of the omentum sewed in between the cut ends to prevent a possible reunion.

4. Exclusion of Pancreatic Juice from the Intestinal Tract.—In one animal two ducts were found, while in another, three ducts were present. Each duct was divided between ligatures and part of the omentum interposed between the cut ends, for the reason just mentioned.

Plan of Investigation.

The general plan of the investigation was to study the daily output of urinary and fecal phenols in dogs under the conditions outlined. The elimination of phenols, under the influence of fasting, catharsis, and phlorhizin, was also studied. Inasmuch as only minimal amounts of phenol were found in the feces—amounts that could have no bearing on the final results—these determinations were dispensed with.

The dogs, kept in metabolism cages, were fed on a standard diet, calorifically sufficient, and containing about 1 gm. of nitrogen per kilo so as to make certain of an excess of food in the intestine. This diet consisted of meat, lard, bread crumbs, sugar, salt, and sufficient bone ash to insure a well formed stool. The food was mixed with 400 cc. of water, and enough additional water was given separately to bring the total daily intake up to about 600 cc. The animals were placed on this diet 3 or 4 days before the

[20] Peet, M. M., *Ann. Surg.*, 1914, lx, 601.

beginning of an experiment. Female dogs only were used, the urine being collected by catheter every morning at the same hour and diluted to 1,000 cc. For a time the volume of urine excreted was noted, but this was later omitted for the reason that no particular relationship was seen between the volume of urine and the amount of phenols eliminated, except as noted in Table I. The body weight was recorded daily immediately after catheterization. Tyrosine was administered *per os*, either suspended in water or mixed with the food. Phenol (Merck reagent) and *p*-cresol (Kahlbaum) were given *per os* in water.

TABLE I.

Eck Fistula. Dog 15–58.

Influence of Water and Eck Fistula on Phenol Excretion.

Date.	Volume of urine.	Total N.	Phenols.				Weight.	Remarks.
			Free.	Total.	Free.	Conjugated.		
1915	*cc.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Oct. 19	600	8.9	0.158	0.181	87	13	12.4	
" 20	910	9.7	0.165	0.192	86	14	12.4	
" 21	695	6.1	0.163	0.188	87	13	12.5	
" 22	910	9.7	0.165	0.190	87	13	12.7	
" 23	745	10.4	0.163	0.188	87	13	12.6	
" 24	550	9.9	0.161	0.186	87	13	12.5	
" 25	415	9.9	**0.173**	**0.203**	**85**	**15**	12.7	Water intake reduced to 300 cc.
" 26	405	9.8	0.177	0.205	86	14	12.7	
" 27	345	9.9	0.173	0.203	85	15	12.8	
" 28	450	9.9	0.174	0.203	86	14	12.9	
" 29	450	9.9	0.172	0.201	86	14	12.9	
" 30	440	9.8	0.172	0.203	85	15	13.0	
" 31	455	9.9	0.170	0.200	85	15	13.0	
Nov. 10								Eck fistula.
" 11	400	10.2	**0.187**	**0.203**	**92**	**8**	12.7	Post-operative condition good.
" 12	250	9.9	**0.186**	**0.204**	**92**	**8**	12.6	
" 13	350	10.3	**0.188**	**0.204**	**92**	**8**	12.9	
" 14	360	10.6	**0.197**	**0.209**	**94**	**6**	13.1	
" 15	590	10.7	**0.205**	**0.213**	**96**	**4**	13.2	
" 16	570	10.6	**0.199**	**0.211**	**94**	**6**	13.1	
" 17	480	10.6	**0.200**	**0.213**	**94**	**6**	13.0	

Eck Fistula.

Experiment A-1.—Dog 15–58 (Table I). This experiment showed several interesting points. The output of phenols from day to day was quite constant, as was also the case in all of our subsequent work. Withdrawing water caused a drop in the volume of urine with a consequent rise in the output of phenols. However, it was seen that where the urine varied normally from day to day, there was little or no effect upon the phenols. After Eck fistula, the free phenols represented from 92 to 96 per cent of the total, the amount of the latter being practically unchanged. On Nov. 17, because of the presence of blood in the urine, the dog was placed on a kennel diet.

Experiment A-2.—Dog 15–58 (Table II). 2 weeks later, on Dec. 1, the regular diet was resumed, the experiment being started Dec. 6. For some reason, which we have been unable to explain —unless it is that the food is digested more rapidly—the absolute amount of total phenols was decreased in the second period, but the free phenols still represented from 96 to 98 per cent of the total. 1 gm. of phenol was fed *per os* with the result that 68.7 per cent of the amount ingested was eliminated. Both free and conjugated phenols were increased. The free phenols represented only 40 per cent of the total, indicating that the large dose of phenol had called forth, to an increased extent, the protective mechanism of the body. A second feeding gave similar results. Feeding 1 gm. of *p*-cresol resulted in the elimination of 50.6 per cent of the amount given. Here also both free and conjugated phenols were increased. The free phenols represented only 31 per cent of the total. Repetition of this feeding gave somewhat similar results. In this experiment and in all subsequent ones, it was noted that the phenol and *p*-cresol administered were eliminated entirely within 24 hours.

The results obtained on feeding tyrosine have a peculiar interest. For example, Dog 15–58 (Table II), receiving 400 gm. of meat—equivalent to 3.20 gm. of tyrosine,[21] or 1.65 gm. of phenol —eliminated daily a total of only 0.159 gm. of phenol. However, on feeding 5 gm. of tyrosine—equivalent to 2.57 gm. of phenol—there was a rise in both free and total phenols, while the

[21] Folin and Denis, *J. Biol. Chem.*, 1912, xii, 246.

TABLE II.

Eck Fistula. Dog 15–58.

Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion after Eck Fistula.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1915	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Dec. 6	9.0	0.143	0.147	97	3	13.9	Cage water resumed.
" 7	9.0	0.147	0.152	97	3	13.9	
" 8	9.1	0.334	0.836 (0.787)* (78.7%)	40	60	14.0	1.000 gm. phenol given in water *per os.*
" 9	9.1	0.152	0.156	97	3		
" 10	9.1	0.153	0.157	97	3	14.2	
" 11	9.2	0.222	0.517 (0.360) (72.0%)	41	59	14.2	0.500 gm. phenol given in water *per os.*
" 12	9.3	0.154	0.159	97	3	14.2	
" 13	9.5	0.156	0.159	98	2	14.4	
" 14	9.5	0.188	0.600 (0.441) (50.6%)	32	68	14.5	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os.*
" 15	9.4	0.154	0.159	97	3	14.5	
" 16	9.5	0.174	0.541 (0.381) (43.8%)	34	66	14.5	*p*-Cresol fed as on Dec. 14.
" 17	9.7	0.154	0.157	98	2	14.5	
" 18	10.3	0.420	0.603 (0.446) (17.7%)	70	30	14.7	5.000 gm. tyrosine (2.570 gm. phenol) given in water *per os.*
" 19	9.6	0.152	0.156	97	3	14.7	

* The figures in the first parenthesis represent "extra" phenols eliminated; those in the second parenthesis denote the output of "extra" phenols in terms of percentage of the amount ingested.

"extra" phenol eliminated was only 0.446 gm., or 17.7 per cent, of the amount ingested. *In other words—and it was to be expected—tyrosine, as present in meat, did not give rise to as much phenol as did free tyrosine. Similarly, phenol, administered as tyrosine or p-cresol, did not give rise to as much phenol as did native*

phenol. It was observed that tyrosine, like phenol and *p*-cresol, is eliminated entirely within 24 hours. A greater conjugation was also seen, though not as large as that produced by phenol and *p*-cresol. A slight rise in the nitrogen elimination was also noted. At no time was it possible to demonstrate unchanged tyrosine, either in the urine or the feces.

Experiment A-3.—Dog 15–58 (Table III). 1 gm. of phlorhizin rubbed up in 10 cc. of olive oil was injected subcutaneously

TABLE III.

Eck Fistula. Dog 15–58.

Influence of Phlorhizin, Fasting, and Catharsis on Phenol Excretion after Eck Fistula.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1915	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Dec. 19	9.6	0.152	0.156	97	3	14.7	
" 20	13.1	0.203	0.277	73	27	14.8	1 gm. phlorhizin.
" 21	15.5	0.217	0.281	77	23	14.7	1 " "
" 22	13.9	0.171	0.210	82	18	14.4	Sugar in urine Dec. 20–23, inclusive.
" 23	9.9	0.154	0.159	97	3	14.3	
							Animal placed on kennel diet till Dec. 31, when regular diet was resumed.
1916							
Jan. 5	10.2	0.150	0.158	95	5	14.7	
" 6	5.2	0.077	0.111	70	30	14.9	Animal fasting; 500 cc. of water given in cage.
" 7	4.8	0.068	0.091	74	26	14.4	
" 8	4.4	0.068	0.092	74	26	13.9	
" 9	4.0	0.068	0.091	75	25	13.6	
" 10	3.6	0.067	0.090	74	26	13.4	
" 11	3.5	0.066	0.091	73	27	13.2	
" 12							3 gm. calomel given; cage urine contaminated; bladder urine contained phenols.
" 13	3.4	0.066	0.089	74	26	12.9	
" 14	3.3	0.066	0.091	73	27	12.7	3 gm. calomel given.
" 25	3.0	0.065	0.089	74	26	11.5	Animal still fasting.

on 2 successive days. The result was an increase in both total and free phenols, the latter representing 73 per cent of the total. Sugar appeared in the urine, just as it did later, under similar cir-

TABLE IV.

Eck Fistula. Dog 16–56.

Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion before and after Eck Fistula.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
May 9	10.3	0.176	0.235	75	25	12.3	
" 10	9.6	**0.385**	**0.782** (0.547) (54.7%)	**49**	**51**	12.4	1.000 gm. phenol given in water *per os.*
" 11	10.1	0.179	0.241	74	26	12.4	
" 12	10.3	**0.435**	**0.606** (0.365) (14.2%)	**72**	**28**	12.5	5.000 gm. tyrosine (2.57 gm. phenol) mixed with food.
" 13	10.2	0.176	0.233	75	25	12.6	
" 14	10.2	**0.210**	**0.556** (0.323) (37.1%)	**38**	**62**	12.5	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os.*
" 15							Eck fistula; post-operative condition good.
" 16	10.1	**0.208**	**0.250**	**83**	**17**	11.9	
" 17	10.2	**0.177**	**0.208**	**85**	**15**	12.1	
" 18	10.3	**0.384**	**0.555** (0.347) (13.5%)	**69**	**31**	12.3	Tyrosine fed as on May 12.
" 19	10.1	**0.350**	**0.815** (0.607) (60.7%)	**43**	**57**	12.5	Phenol fed as on May 10.

cumstances, in a normal dog. This is in accord with the observations of Sweet and Ringer,[22] who found that upon giving phlorhizin to a dog with Eck fistula, a glycosuria resulted quite comparable to that occurring in a normal dog.

[22] Sweet, J. E., and Ringer, A. I., *J. Biol. Chem.*, 1913, xiv, 135.

In fasting, as was to be expected, there was a diminution in both total and free phenols, the latter amounting to 74 per cent of the total. A greater ability to conjugate was shown, but no initial fall and subsequent rise in the phenol excretion as described by Herter[4] and Müller[5] was noted. Neither was it possible, by giving calomel, to rid the urine entirely of phenols. In this connection, it is interesting to recall that Baumann,[13] owing perhaps to the inadequacy of the prevailing methods, could get no test for phenols after administering calomel to a fasting dog.

Experiment G.—Dog 16–56 (Table IV). The results of this experiment are in accord with those obtained in the previous Eck fistula dog. After the operation there was a tendency for the total phenol elimination to decrease, due perhaps to a more rapid rate of digestion. The influence of tyrosine before and after the operation is about the same—if anything, for the reason just mentioned, less total phenols are obtained from the same amount of tyrosine after the operation than before.

Influence of Tyrosine, Phenol, and p-Cresol on Phenol Excretion in Normal Dogs.

Experiments K and M.—Dogs 15–74 and 16–20 (Table V). In general the findings here corroborated those of Experiment G.

TABLE V.

Normal Dog 15–74.

Influence of Tyrosine on Phenol Excretion.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1915	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Nov. 21	12.1	0.187	0.217	86	14	14.3	
" 22	7.4	0.187	0.217	86	14	14.4	
" 23	12.1	**0.447**	**0.610** (**0.393**) (**15.3%**)	**73**	**27**	14.4	5.000 gm. tyrosine (2.570 gm. phenol) given in water *per os.*
" 24	12.8	0.187	0.217	86	14	14.7	
" 25	13.2	0.189	0.217	87	13	14.8	

TABLE V—*Concluded.*

Dog 16–20.

Influence of Phenol and p-Cresol on Phenol Excretion.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Feb. 20	10,5	0.158	0.192	83	17	11.1	
" 21	6.3	0.164	0.195	84	16	11.3	
" 22	10.7	0.167	0.196	85	15	11.1	
" 23	10.5	0.169	0.196	86	14	11.1	
" 24	10.2	0.166	0.192	86	14	11.1	
" 25	9.9	**0.181**	**0.647** (**0.455**) (**52.3%**)	**41**	**59**	11.2	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os.*
" 26	10.1	0.157	0.192	82	18	11.3	
" 27	10.2	**0.417**	**0.886** (**0.694**) (**69.4%**)	**47**	**53**	11.4	1.000 gm. phenol given in water *per os.*

Exclusion of Pancreatic Juice from the Intestinal Tract.

Experiment B-1.—Dog 15–63 (Table VI). The normal figures recorded here are in accord with those of Tables II and V.

Experiment B-2.—Dog 15–63 (Table VII). This experiment corroborates fully Experiment A-3 (Table III). While the results in the latter experiment were observed in a dog with Eck fistula, those of the present experiment were noted in a normal dog.

Experiment B-3.—Dog 15–63 (Table VIII). Several differences were noted between the results of this experiment and those of Experiment B-2 (Table VI). After the operation, the total amount of phenols eliminated was increased, resulting in a greater conjugation. On administering tyrosine, a greater percentage was eliminated as phenols. Undoubtedly this was due to the fact that the absence of pancreatic juice from the intestinal tract retarded the processes of digestion. The administration of phenol and of *p*-cresol resulted in a lessened elimination; *i.e.*, with phenol, there was a drop from 75.8 per cent to 51.1 per cent of

TABLE VI.

Exclusion of Pancreatic Juice. Dog 15–63.

Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion before Operation.

Date.	Total N.	Phenols. Free.	Phenols. Total.	Free.	Conjugated.	Weight.	Remarks.
1915	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Dec. 6	12.7	0.189	0.223	85	15	15.1	
" 7	12.6	0.192	0.222	86	14	15.1	
" 8	13.1	**0.442**	**0.980 (0.758) (75.8%)**	**45**	**55**	15.2	1.000 gm. phenol given in water *per os.*
" 9	12.9	0.195	0.228	86	14		
" 10	13.1	0.195	0.226	86	14	15.3	
" 11	12.9	**0.411**	**0.944 (0.718) (71.8%)**	**44**	**56**	15.4	Phenol fed as on Dec. 8.
" 12	13.0	0.195	0.228	86	14	15.2	
" 13	13.9	0.195	0.224	87	13	15.5	
" 14	14.2	**0.215**	**0.651 (0.427) (49.2%)**	**33**	**67**	15.5	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os.*
" 15	14.1	0.195	0.225	87	13	15.5	
" 16	14.2	**0.218**	**0.606 (0.381) (43.8%)**	**36**	**64**	15.4	Cresol fed as on Dec. 14.
" 17	13.5	0.195	0.223	87	13	15.6	
" 18	14.5	**0.455**	**0.625 (0.402) (15.6%)**	**73**	**27**	15.6	5.000 gm. tyrosine (2.57 gm. phenol) given in water *per os.*
" 19	13.3	0.192	0.222	87	13	15.7	

TABLE VII.

Exclusion of Pancreatic Juice. Dog 15–63.

Influence of Phlorhizin, Fasting, and Catharsis on Phenol Excretion before Operation.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1915	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Dec. 19	13.3	0.192	0.222	87	13	15.7	
" 20	14.8	0.200	0.268	75	25	15.8	1 gm. phlorhizin.
" 21	15.5	0.212	0.278	76	24	15.7	1 " "
" 22	14.5	0.192	0.232	83	17	15.6	Sugar in urine Dec. 20–23, inclusive. Animal placed on kennel diet till Dec. 31, when regular diet was resumed.
1916							
Jan. 5	13.4	0.188	0.224	84	16	16.0	
" 6	4.6	0.090	0.130	70	30	16.0	Animal fasting; 500 cc. of water given in cage.
" 7	3.6	0.081	0.107	76	24	15.7	
" 8	3.5	0.080	0.106	76	24	15.4	
" 9	3.2	0.080	0.106	76	24	15.2	
" 10	3.1	0.079	0.106	75	25	14.9	
" 11	3.3	0.081	0.107	76	24	14.8	
" 12							3 gm. calomel given; cage urine contaminated; bladder urine contained phenols.
" 13	3.1	0.080	0.106	76	24	14.3	
" 14	3.0	0.081	0.106	76	24	14.1	3 gm. calomel given.
" 26	11.1	0.184	0.223	83	17	15.2	Animal on regular diet since Jan. 15.

TABLE VIII.

Exclusion of Pancreatic Juice. Dog 15–63.
Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion after Operation.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Jan. 26	11.1	0.184	0.223	83	17	15.2	
" 28							Pancreatic ducts cut; dog in good condition.
Feb. 6	9.4	0.200	0.289	69	31	15.5	
" 7	10.0	0.203	0.293	69	31	15.4	
" 8	9.7	0.202	0.286	71	29	15.3	
" 9	9.9	0.660	0.845 (0.559) (21.7%)	78	22	15.4	5.000 gm. tyrosine (2.57 gm. phenol) mixed with food.
" 10	9.5	0.202	0.289	70	30	15.3	
" 11	9.5	0.200	0.293	68	32	15.5	
" 12	10.1	0.279	0.632 (0.339) (38.9%)	44	56	15.5	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os.*
" 13	8.7	0.203	0.289	70	30	15.3	
" 14	8.5	0.423	0.800 (0.511) (51.1%)	53	47	15.3	1.000 gm. phenol given in water *per os.*
" 15	8.6	0.200	0.286	70	30	15.2	

the amount fed; with *p*-cresol, there was a drop from 49.2 per cent to 38.9 per cent of the amount given. With both substances there is, however, an increased conjugation because of the increased formation of phenols.

Experiment H.—Dog 16–55 (Table IX). The results of this experiment need no further comment since they corroborate the previous findings as recorded in Tables VI and VIII.

TABLE IX.

Exclusion of Pancreatic Juice. Dog 16–55.
Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion before and after Section of Pancreatic Ducts.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
May 9	9.5	0.151	0.200	75	25	11.6	
" 10	9.8	**0.417**	**0.847** (0.647) (64.7%)	**49**	**51**	11.6	1.000 gm. phenol given in water *per os.*
" 11	9.6	0.156	0.204	76	24	11.7	
" 12	9.6	**0.208**	**0.594** (0.390) (44.8%)	**35**	**65**	11.8	1.000 gm. cresol (0.870 gm. phenol) given in water *per os.*
" 13	9.7	0.153	0.200	76	24	11.8	
" 14	9.8	**0.440**	**0.625** (0.425) (16.5%)	**70**	**30**	11.8	5.000 gm. tyrosine (2.570 gm. phenol) mixed with food.
" 15							Pancreatic ducts cut; dog in poor condition.
" 16	9.6	**0.175**	**0.250**	**70**	**30**	11.3	
" 17	9.4	**0.173**	**0.255**	**68**	**32**	11.0	
" 19							Animal chloroformed.

Intestinal Obstruction.

Experiment C.—Dog 16–6 (Table X). The results obtained before the operation are in accord with those found in normal dogs. After the operation, it is worthy of note that although the animal ate practically nothing, the formation and elimination of phenols rose to a high level, the free phenols representing only 56 per cent of the total. In other words, the conjugation, due to the larger amounts of phenols present, was increased just as though a dose of phenol had been ingested. It was noted also that on giving phenol after the operation, only 51 per cent was eliminated, while before the operation the output was 64 per cent. This is explained on the ground that digestion is markedly retarded. In this respect the results were somewhat similar to

TABLE X.

Intestinal Obstruction. Dog 16–6.

Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion before and after Intestinal Obstruction.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Feb. 23	9.6	0.175	0.227	77	23	10.9	
" 24	9.5	0.171	0.222	77	23	11.1	
" 25	9.7	0.199	0.548 (0.326) (37.4%)	36	64	11.1	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os*.
" 26	9.6	0.173	0.221	78	22	11.2	
" 27	9.5	0.400	0.862 (0.641) (64.1%)	46	54	11.1	1.000 gm. phenol given in water *per os*.
" 28	9.7	0.174	0.221	79	21	11.3	
" 29	9.8	0.429	0.605 (0.384) (15.0%)	71	29	11.4	5.000 gm. tyrosine (2.57 gm. phenol) mixed with food.
Mar. 1	9.9	0.173	0.219	79	21	11.3	
" 9							Intestine obstructed.
" 10							Urine contaminated.
" 11							" "
" 23	9.8	0.263	0.477	55	45		Dog defecated hard stool for first time since operation.
" 24	7.2	0.245	0.442	56	44	9.6	Ate only part of diet.
" 25	6.4	0.238	0.403	58	42	9.2	No food given Dec. 25 and Dec. 26.
" 26	4.9	0.500	0.914 (0.511) (51.1%)	55	45	8.9	1.000 gm. phenol given in water *per os*.

those obtained before and after the exclusion of pancreatic juice from the intestinal tract. On March 27, when the animal was chloroformed, the intestine was found to be dilated to about 100 times the normal capacity for about 3 feet above the obstruction; this dilatation continued in a lesser degree up to the jejunum. The jejunum and duodenum were normal. It would appear that almost complete obstruction had been obtained.

Experiment F.—Dog 15–63 (Table XI). The findings in this experiment were not as marked as those in Experiment C, due perhaps to the fact that the amount of obstruction secured in this case was not as great as in Dog 16–6. However, there was a definite rise in the formation and elimination of phenols, though nothing unusual was to be noted as a result of the combination of pancreatic insufficiency and intestinal obstruction. Apparently the increased phenol formation was not sufficient to cause an increase in the conjugation, the free phenols representing almost the same per cent of the total both before and after the operation.

TABLE XI.

Dog 15–63.

Influence of Intestinal Obstruction, Combined with Pancreatic Insufficiency, on Phenol Excretion.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Apr. 8	12.7	0.190	0.270	70	30	13.9	
" 9	12.9	0.200	0.263	76	24	14.2	
" 10	13.2	**0.498**	**0.860 (0.597) (59.7%)**	**58**	**42**	14.2	1.000 gm. phenol given in water *per os.*
" 11	12.9	**0.357**	**0.588 (0.325) (37.3%)**	**61**	**39**	14.3	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os.*
" 12	12.8	0.187	0.257	72	28	14.3	
" 13	12.7	0.195	0.263	74	26	14.3	
" 17							Intestine obstructed.
" 19	7.5	**0.227**	**0.316**	**71**	**29**	13.7	Dog did not eat.
" 20	6.5	**0.543**	**0.870 (0.554) (55.4%)**	**63**	**37**	13.5	" " " " 1.000 gm. phenol given in water *per os.* No lard in diet beginning Apr. 22.
May 3	10.9	**0.223**	**0.307**	**72**	**28**	13.7	
" 4	11.4	**0.555**	**0.855 (0.548) (54.8%)**	**65**	**35**	13.8	Phenol fed as on Apr. 20.
" 5	11.3	**0.227**	**0.316**	**72**	**28**	13.7	

Exclusion of Bile from the Intestinal Tract.

Experiment D.—Dog 16–39 (Table XII). The figures obtained before the operation are similar to those in a normal dog. After the operation, however, a striking difference was noted. *Although*

TABLE XII.

Exclusion of Bile. Dog 16–39.

Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion before and after Operation.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Mar. 27	13.6	0.161	0.215	75	25	19.6	
" 28	13.8	0.365	0.758 (0.543) (54.3%)	48	52	19.9	1.000 gm. phenol given in water *per os.*
" 29	13.7	0.167	0.217	76	24	20.3	
" 30	13.9	0.447	0.613 (0.396) (14.8%)	72	28	20.5	5.000 gm. tyrosine (2.570 gm. phenol) mixed with food.
" 31	13.9	0.189	0.590 (0.373) (42.8%)	32	68	20.7	1.000 gm. *p*-cresol (0.870 gm. phenol) given in water *per os.*
Apr. 1							Bile duct cut; dog in good condition.
" 2	10.9	0.167	0.208	80	20	20.5	Bile in urine; little food eaten.
" 3	10.7	0.330	0.642 (0.434) (43.4%)	52	48	20.6	Phenol fed as on Mar. 28; dog ate very little; bile in urine.
" 7							Dog gave birth.
May 4	5.8	0.238	0.291	82	18	11.8	Animal markedly jaundiced.
" 5	6.4	0.291	0.513 (0.222) (25.5%)	57	43	11.5	Cresol fed as on Mar. 31.
" 6	6.5	0.263	0.329	80	20	11.4	
" 9	6.7	0.629	0.839 (0.510) (19.8%)	75	25		Tyrosine fed as on Mar. 30.

there was an increase in the formation and elimination of phenols, the free phenols represented 80 per cent of the total, as against 75 per cent before the operation. In all other experiments, an increase in the phenol formation was accompanied by a decrease in the output of free phenols, figured as per cent of total, while in this experiment and in the following one the reverse was the case. Of the ingested phenol and *p*-cresol, 43.3 per cent of the former and 25.5 per cent of the latter were eliminated after the operation as against 54.3 and 42.8 per cent, respectively, before. Feeding tyrosine caused an increased conjugation, the free phenols dropping from 75 to 72 per cent before the operation, and from 80 to 75 per cent after. It was observed, however, that only 14.8 per cent of the ingested

TABLE XIII.

Exclusion of Bile. Dog 16–41.
Influence of Phenol, p-Cresol, and Tyrosine on Phenol Excretion before and after Operation.

Date.	Total N.	Phenols.				Weight.	Remarks.
		Free.	Total.	Free.	Conjugated.		
1916	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*	
Apr. 8	11.3	0.151	0.191	79	21	9.6	
" 9	11.1	0.152	0.186	82	18	9.7	
" 10	11.2	0.477	0.782 (0.596) (59.6%)	61	39	9.7	1.000 gm. phenol given in water *per os.*
" 11	11.2	0.347	0.568 (0.382) (43.8%)	61	39	9.7	1.000 gm. cresol (0.870 gm. phenol) given in water *per os.*
" 12	11.1	0.147	0.182	81	19	9.8	
" 13	9.0	0.440	0.596 (0.314) (12.2%)	74	26	9.9	5.000 gm. tyrosine (2.57 gm. phenol) given in water *per os.*
" 14							Bile duct cut; animal in poor condition.
" 15	5.6	0.098	0.123	80	20	8.9	No food eaten; bile in urine.
" 16	5.2	0.096	0.120	80	20	8.9	No food eaten; bile in urine.
" 17							Animal chloroformed.

tyrosine was eliminated before the operation as against 19.8 per cent after. This was similar to the result obtained with tyrosine after excluding the pancreatic juice, and, as in the latter case, may be explained on the basis of delayed digestion.

Experiment E.—Dog 16-41 (Table XIII). The results of this experiment were not as clean cut as they might be, for the animal refused to eat and was rather sick. On autopsy the bile duct, up to the point tied, was greatly enlarged.

DISCUSSION.

The formation of phenolsulfuric acids is one example of the ability of the body to convert poisonous substances into harmless compounds. It is, however, an open question as to whether this power of the body is sufficient for all purposes. Since the time of Baumann it has been believed that phenols were quantitatively converted into harmless phenol esters; hence phenols were estimated on the basis of conjugated sulfates, whereas our figures corroborate the statement made by Folin[1] that the greater part of the phenols are excreted unconjugated.

It has been thought that the free phenols are harmful to the body, and this must be due primarily to an increase of total phenols, with its accompanying increase of free phenols. For example, it is seen in normal cases that the absolute amount of free phenols represents from 75 to 85 per cent of the total. On the other hand, where there is a rise in total phenols, whether due to ingested phenols or to some pathological condition, the free phenols, although increased absolutely, represent from 30 to 70 per cent of the total. In other words, the absolute amount of free phenols should be taken into consideration together with the percentage in considering their deleterious influence.

With one exception, an increase in the formation of phenols, whether normal or pathological, resulted in an increased conjugation, though the reverse might have been expected. It would appear that the protective mechanism of the body responded to the greater stimulus. The exception noted was in the case where bile was excluded from the intestinal tract. Here there was an increased phenol formation accompanied by a decreased conjugation. It would seem, therefore, that the bile plays some part in assisting the liver in its conjugating function.

There is a greater conjugation after feeding *p*-cresol than after giving phenol. In view of the fact that *p*-cresol makes up the larger part of the urinary phenols, it seems plausible to assume that when it is introduced into the body it is more easily conjugated. Hence on feeding this substance the elimination of free phenols rises only very little.

The high percentage of free phenols prevailing after Eck fistula was to be expected, inasmuch as the liver, which is the main seat of conjugation, has been cut out of the circulation. In spite of this, upon the ingestion of phenol there is an increased conjugation, showing that other organs, when necessary, can take up the work of the liver in this respect.

Lewin[6] held that the increased protein breakdown accounted for the increased phenol formation when phlorhizin was given. While it is true that phlorhizin causes a protein breakdown, it is highly improbable that this is the reason for an increased phenol production. Regarding the fate of phlorhizin in the body, it has been shown[23] that when injected, part of it is eliminated as a combined glucoronic acid while another part apparently undergoes further change; also, unchanged phlorhizin can be found for some time in the blood and tissues. In view of all this, when it is considered that phlorhizin contains two benzene radicals, and that benzene when fed[1] gives rise to phenol, it seems likely that it is the phlorhizin *per se* which is converted into phenol.

The results obtained show, among other things, that gastro-intestinal disturbances, in which it is reasonable to assume that there is an increase in the formation of phenol, and also certain liver diseases, where it is supposed that the mechanism for the detoxication of phenols has been impaired, offer a field for investigation, for, as Folin[1] points out, and as this work proves, the phenols can be taken as an index of intestinal putrefaction.

SUMMARY.

The results of this investigation, in addition to confirming the findings of Folin,[1] bring to light some interesting observations.

A. 1. The elimination of phenols from day to day is quite constant.

[23] Von Fürth, O., Chemistry of Metabolism, Philadelphia, 1916, 280.

2. Withdrawing water from the diet causes an increased phenol elimination.

3. After Eck fistula, the free phenols represent as high as 97 per cent of the total, the latter, as also the former, exhibiting a tendency to decrease.

4. After intestinal obstruction, there is an increase in both free and total phenols, the former constituting as low as 55 per cent of the latter.

5. In pancreatic insufficiency, there is an increase in both free and total phenols with an accompanying decrease in the percentage of free phenols.

6. After excluding the bile, both free and total phenols increase, but with an increase in the percentage of free phenols.

B. 1. The feeding of phenol and *p*-cresol results normally, with but slight variations, in the elimination of about 65 per cent and 40 per cent respectively.

2. After Eck fistula, about the same excretion is noted.

3. After intestinal obstruction, pancreatic insufficiency, and exclusion of bile, there is in both cases a drop in the amount excreted.

C. 1. The feeding of tyrosine results normally in an excretion of about 14 per cent, as phenols.

2. After Eck fistula, practically the same amount is eliminated.

3. After exclusion of bile and pancreatic juice, about 20 per cent of ingested tyrosine is eliminated.

D. Feeding of any of the three substances causes an increase in the conjugation at all times—*p*-cresol to a greater degree than phenol, and the latter to a greater extent than tyrosine.

E. Tyrosine, phenol, and *p*-cresol, fed in amounts of 5 gm., 1 gm., and 1 gm. respectively, were all eliminated within 24 hours.

F. No unchanged tyrosine could be demonstrated in the urine or feces.

G. Fasting reduces the phenols to a low level; the injection of phlorhizin during fasting causes an increase in the output of phenols.

H. It is impossible to free the urine entirely of phenols by the use of calomel.

I. The bile appears to have some influence on the conjugating function of the liver.

J. The phenols can be taken as an index of intestinal putrefaction.

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